PREDATION OF *NEOPLASTA* COQUILLETT LARVAE (DIPTERA: EMPIDIDAE) ON LARVAL MIDGES IN THE GENUS *RHEOTANYTARSUS* BAUSE (DIPTERA: CHIRONOMIDAE)

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Abstract.—Larvae of in the genus *Neoplasta* Coquillett were systematically collected from midge tubes in a Southern California mountain stream. From October 1993 through August 1996, 4234 tubes of *Rheotanytarsus* Bause species (Diptera: Chironomidae) were examined and predatory *Neoplasta* larvae were found in 1.75% of the tubes. The peak occurrence of the predator was during August to February. A significant negative correlation (cc = −0.445) between the presence of midges and *Neoplasta* larvae in the tubes suggests that these predators have a significant impact on the midge population. The predatory behavior and molting patterns were described from laboratory rearing of the *Neoplasta* larvae.

Key Words.—Insecta, Empididae, *Neoplasta,* Chironomidae, *Rheotanytarsus,* predation, stream

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MacDonald & Turner (1993) reviewed the systematics of the genus *Neoplasta* Coquillett (Diptera: Empididae: Hemerodromiinae) north of Mexico, expanding the number of species in the region from three to twelve. Except for this study, the biology of the genus remains poorly known. A limited number of observations exist about the adults of *Neoplasta*. Peterson (1960) reported that adults of *Neoplasta scapularis* (Loew) "probed at larvae of *Simulium piperi*" along the margins of a Utah stream. Harper (1980) reported the preference of *N. scapularis* for swift water in adult emergence studies in Québec. Based on adult emergence patterns, Landry & Harper (1985) suggest that immatures of *N. scapularis* are found in small as well as large streams in Québec and that adults have a relatively long emergence period as compared to other aquatic empidids. Similar emergence patterns were reported by MacDonald & Turner (1993). The pupal stage of a *Neoplasta* species near *brevicornis* Collin was described by Knutson & Flint (1971) from the pupal cases of Tricopterans in a Chilean stream. The larval stage of *Neoplasta* was recently described by MacDonald & Harkrider (1999).

The predaceous habits of related larvae were established by Vaillant (1953), who found larvae of *Hemerodromia sequyi* Vaillant feeding on *Simulium* Latreille species larvae. Other studies, such as Hamada (1993), have shown an association between *Hemerodromia* Meigen larvae and *Simulium perflavum* Roubaud. Except for these studies, and a few incidental reports, no information exists for larvae in the subfamily Hemerodromiinae. Because the adults of aquatic dance flies are often common (Harper 1980), and both adult and larval stages have described as predaceous on the medically important Simuliidae, a closer investigation of this group seems appropriate to evaluate for their biological control potential.

This study focuses on larvae of the *Neoplasta hebes* Melander complex that occur in tubes of larval *Rheotanytarsus* Bause (Diptera: Chironomidae) in a Southern California stream. The seasonal occurrence, effects on the host population, and observations on reared larvae are documented.

Materials and Methods

Study Site.—Systematic sampling was conducted along a first-order stream in the San Gabriel Mountains of Southern California. The site was located above the west-bank of San Antonio Creek where the water runs parallel to the creek through alluvial deposits. The elevation was approximately 1300 m. The site was located 300–350 m north of the highway bridge crossing the creek on the north side of Baldy Village (34°14'25" N, 117°39'10" W). The surrounding vegetation was dominated by alder (*Alnus rhombifolia* Nuttall) and canyon oaks (*Quercus chrysolepis* Liebmann). An unusual feature of this site was the presence of numerous California laurel trees (*Umbellularia californica* Nuttall) along and hanging over the water course. The permanent spring provides water at 13°–16° C, depending on the season. Throughout most of the study, samples were taken at two sites. Site A was characterized by a rocky riffle immediately below a small pool. Moss and other vegetation were common at this site. Site B, located 40 m below site A, was along a rocky channel with little vegetation. Due to a partial divergence of the water caused by a series of landslides beginning in April 1995, a third site C was established approximately 30 m above site A. This site was similar to site A.

Sampling.—Stone samples were collected at random by hand at the collection sites and late-instar larval tubes of *Rheotanytarsus* were systematically removed using forceps. Samples were put in a plastic, wide-mouthed container and kept in a cooler on ice for return to the laboratory. Each tube was dissected in the laboratory using #3 insect pins on the same day as collected. The presence of empidid larvae, *Rheotanytarsus* larvae or pupae was recorded. Even though stored on ice, some larval midges escaped their tubes during transport. These individuals were counted as inhabitants in data calculations. The midge inhabitants were calculated as the percentage of the number of midge larvae and pupae relative to the number of tubes collected.

Empidid larvae that were damaged during collection or dissection were fixed in 70% ethyl alcohol and later processed through 95% ethyl alcohol into Euparol for mounting on microscope slides. Measurements of empidid larvae head capsules were made with an ocular micrometer at magnification of 400×. The length of the scleritized portion of the head was measured. Undamaged larvae were reared in 250 ml containers with coarse sand and aeration. *Rheotanytarsus* sp. larvae and numerous organisms from the collecting site were provided as prey.

Some late-instar *Rheotanytarsus* sp. larvae were reared to adults in emergence units similar those used to rear empidids. Adult midges were preserved in 70% ethyl alcohol.

Sampling began in October 1993 and continued at roughly monthly intervals through August 1996. During the period of January 1994 to December 1994, systematic sampling was conducted at both sites A and B. A minimum of 75 tubes were taken from each site at monthly intervals. When the population was low, tubes were collected for an hour at each site. In April 1994, no midge tubes were found at site A.

Results

Prey Populations.—Reared specimens of *Rheotanytarsus* species were identified as New Species 2 (6 individuals) and New Species 3 (4 individuals) by
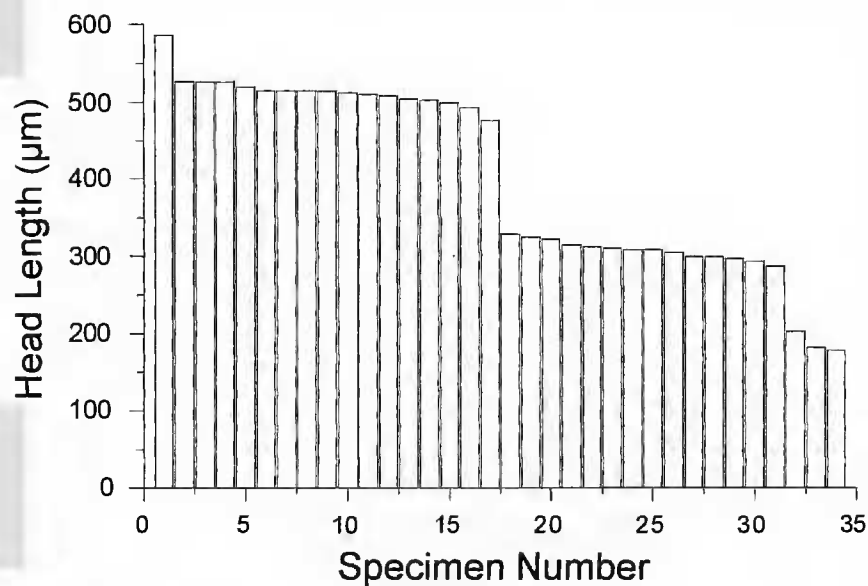
Figure 1. Head measurements (scleritized region) of larval empidids collected from *Rheotanytarsus* tubes.

Sublette (personal communication). These species are to be named in a revision of this genus. Because the sample cohorts sent for examination were small, other species may occur at the site which were missed in my samples.

Empidid Larvae.—Larvae were identified as *Neoplasta* after rearing to the adult stage. The empidid larvae collected in this study are described in MacDonald & Harkrider (1999).

The empidid larvae found in the *Rheotanytarsus* tubes were primarily in the *Neoplasta hebes* complex. A single, female specimen of *Neoplasta scapularis* (MacDonald & Turner 1993) was reared (specimen 1 in Fig. 1). The *N. scapularis* larval head capsule was larger than the others (585 μm), reflecting the larger size of *N. scapularis* adults. Four of the measured specimens (2–5 in Fig. 1) were exuviae of reared larvae. The reared specimens were females of the *N. hebes* complex. Specific identification is not possible from females (MacDonald & Turner 1993). A group of 16 larvae (specimens 2–17 in Fig. 1) were nearly equal in head size (mean = 511 ± 6.6 μm), suggesting that this cohort represents the last instar larvae of the *N. hebes* complex. The exuvia cataloged as specimen 19 (Fig. 1) molted to specimen (6). Sample 19 was the penultimate larval instar of *N. hebes* complex. A large group of specimens (18–31) are similar in head capsule size (mean = 307 ± 7.1 μm) and are the penultimate larval instars of the *N. hebes* complex. The last three specimens represent early instars. These mounted specimens indicate that three separate instars are found in the *Rheotanytarsus* tubes in this location and that most are in the *N. hebes* complex, probably *Neoplasta parahebes* MacDonald & Turner. More recent studies indicate that approximately 78% of the adult Hemerodromiinae collected along this stream were *N. parahebes* with less than 1% *N. hebes* (Harkrider 2000).

Field Population.—The relative number of midge tubes inhabited by *Rheotanytarsus* larvae and pupae during the systematic sampling of the two sites is shown in Fig. 2. A Spearman correlation between the midge populations showed a correlation between the midge inhabitants at the two sites (cc = 0.576, P = 0.039), but little correlation existed between the *Neoplasta* populations (cc = 0.263, P = 0.385). *Neoplasta* larvae were more numerous at site B (2.4% of tubes versus 1.6% of tubes at site A) suggesting that *Neoplasta* larval predation may have a
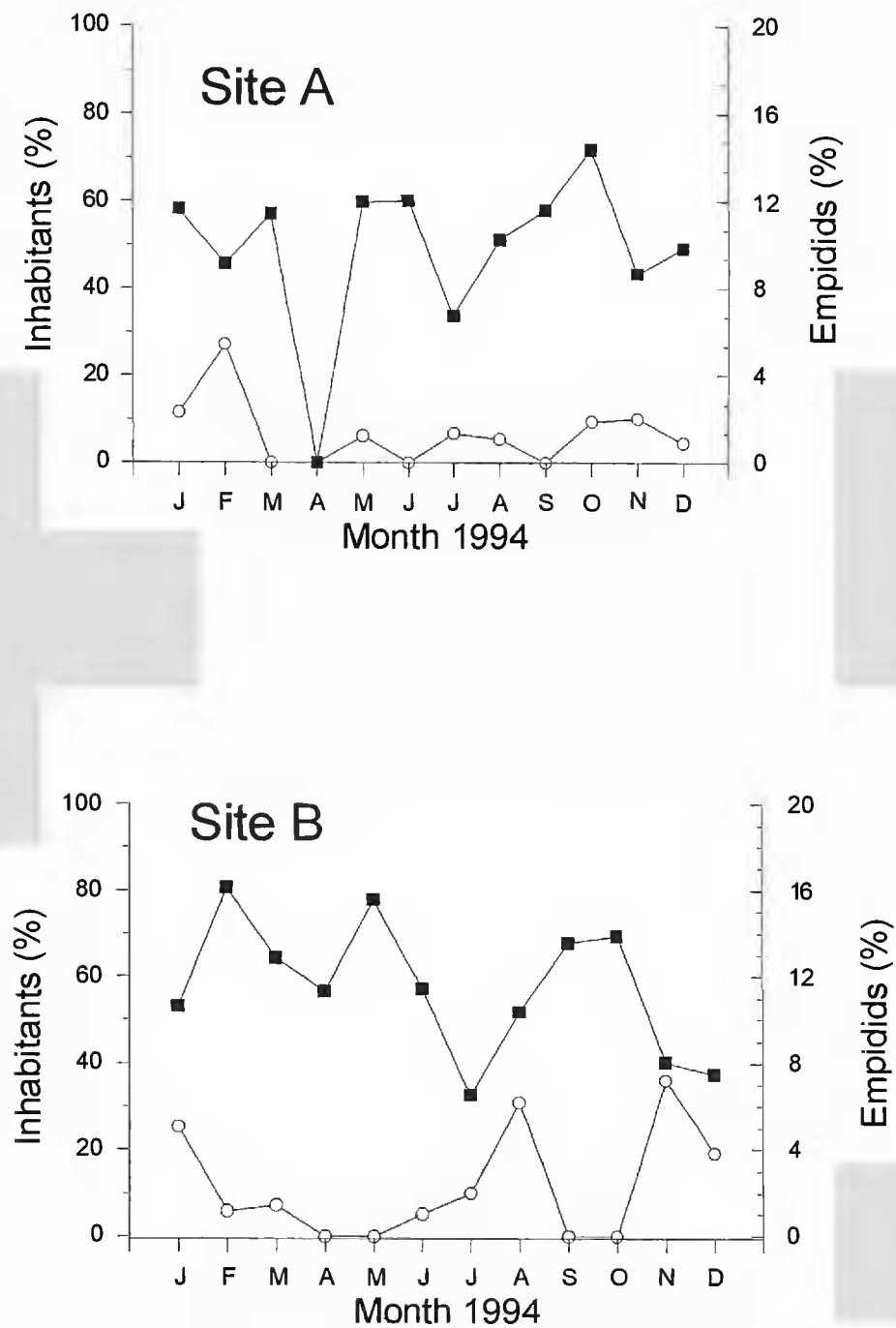
Figure 2. The percentage of *Rheotanytarsus* tubes occupied by immature midges (squares) and empidid larvae (circles) collected during systematic sampling of a site below a rock pool (A) and a rocky channel (B).

more significant impact on the midge population at site B. The Spearman correlation coefficient between the number of *Neoplasta* larvae and the percentage midge inhabitants at site A (cc = −0.349, P = 0.27) was not significant but the correlation coefficient between the *Neoplasta* and midge populations at site B was highly significant (cc = −0.693, P = 0.006).

An analysis of the number of tubes inhabited by midges verses the number of empidids for all the sampling sites (Fig. 3) shows a highly significant negative correlation of cc = −0.445 (P = 0.0017).

A compilation of the monthly habitation rates for midges and *Neoplasta* larvae is shown in Table 1. The most striking feature of this distribution is the scarcity of larval *Neoplasta* from April through June.

Feeding Behavior.—Most reared *Neoplasta* larvae died within a day or two of collection. Larvae that survived the first two days, but did not feed, died within a week. Of those that fed, the prey was restricted to chironomid larvae in the tribe Tanytarsini, i.e., *Rheotanytarsus* species from the streams and *Paratanytar-*
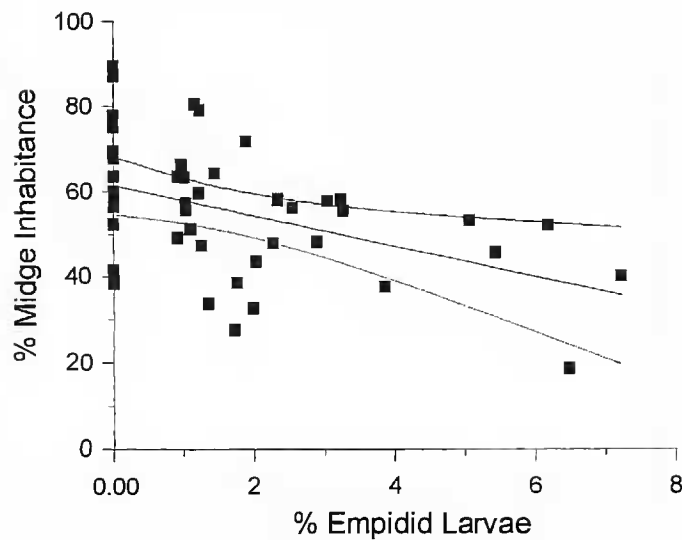
Figure 3. The percentage of immature midges in tubes relative to the percentage empidid larvae is shown for 41 samples from the three Baldy Village sites. A regression line is shown with a 95% confidence interval. The Spearman correlation coefficient was cc = − 0.445 (P = 0.0017).

sus grimii (Schneider) from a laboratory colony. Numerous organisms from the host environment were made available in the rearing units including nematodes, oligochetes, ostracods, and black fly larvae. Various sized larvae of *Chironomus* sp. and *Dicrotendipes californicus* (Johannsen) (Chironomidae) were often presented in rearing units, but none stimulated the *Neoplasta* larvae to feed.

Feeding behavior of the *Neoplasta* larvae could be best characterized as stalking. On a few occasions, when presented midge larvae, the larvae would strike and feed outside the midge larval tube. Usually, however, the *Neoplasta* larva would hesitatingly approach an occupied midge tube from the side or rear. The *Neoplasta* larvae would enter the rear of the tube or explore the outside. As the empidid neared the prey, it would approach with the head retracted. Often the *Neoplasta* larva would freeze with the retracted head and thorax slightly raised while the prolegs became firmly attached to the silk of the tube. The empidid would strike quickly, in a snake-like fashion, with the mouth parts attaching to the cuticle of the prey. Usually, the attack was on one of the back abdominal

Table 1. A summary of the total collection (by month) at three Baldy Village collection sites from October, 1993, through August, 1996.

Month	Tubes sampled	Tubes inhabited (%)	Empidids (% tubes)
January	338	175 (51.8)	9 (2.7)
February	470	293 (62.3)	13 (2.8)
March	341	182 (53.4)	6 (1.8)
April	347	208 (59.9)	2 (0.6)
May	346	236 (68.2)	1 (0.3)
June	260	138 (53.1)	1 (0.4)
July	215	97 (45.1)	3 (1.4)
August	210	104 (49.5)	6 (2.9)
September	410	215 (52.4)	13 (3.2)
October	381	239 (62.7)	4 (1.0)
November	382	159 (41.6)	11 (2.9)
December	534	246 (46.1)	7 (1.3)
Total	4234	2292 (54.1)	76 (1.79)

segments of the prey, if the predator had entered the back of the midge tube, or occasionally on a thoracic or forward abdominal segment, if the attack was made through the tube or from the mouth of the larval tube.

When struck, the midge larva would stop or greatly reduce movement. Typically, the midge's body would elongate and become rigid with occasional twitching of the prey's prolegs and mandibles. The head of the *Neoplasta* larvae would move into the hemocoel of the prey while the mandibles and maxillae probed tissues. The body of the prey would become liquefied and the peristaltic movements of the empidid gut would gradually accumulate a greenish liquid in the midgut. Often, the feeding would continue systematically throughout the body of the prey, leaving only the cleared cuticle of the prey. This took over 4 h for a medium sized midge larva. This behavior pattern suggests that the larva paralyses the prey with a neurotoxin and proceeds to feed by extra-oral digestion (Cohen 1998). In some cases, the newly fed *Neoplasta* larvae would remain in the midge tube for hours or days after feeding.

In the laboratory, active *Neoplasta* larvae killed one or more midge larvae per day. In one successful rearing, a *N. hebes* complex larva fed on 45 midge larvae in 31 days (1.45 larvae/day). In field collections, *Neoplasta* larvae were observed to feed on both larvae and pupae.

Molting and Pupation.—In several instances, *Neoplasta* larvae moved into a black fly (Simuliidae) pupal case, with or without the black fly pupa being in the case. In one case the crochets of the prolegs locked the *Neoplasta* larva to the silk of the pupal cases and it proceeded to molt to the next larval instar. There was no evidence that the *Neoplasta* larva fed on the black fly pupae. *Neoplasta* larvae do not feed for several days before molting or pupating. The non-feeding larvae typically remain quiescent in a midge tube or the sandy substrate. In the laboratory all pupation and adult emergence took place in the sand. The pupal stage took from 9 to 11 d under laboratory conditions.

DISCUSSION

This study establishes *Rheotanytarsus* species as a prey for larvae of the *N. hebes* complex. The predacious larvae were observed feeding on both midge larvae and pupae in field collected material, and numerous observations were made during laboratory rearings. Midge tubes were also used as resting sites after feeding, as numerous field collected larvae were engorged at the time of collection. Similar behavior was observed in the laboratory. The relative role of *Rheotanytarsus* species as prey for empidid larvae remains problematic. Because this study focused on a very restricted habitat, the proportion of the predator population in the study area that was actually sampled can not be known. The midge tubes may be one of several habitats in which the empidid larvae find prey. The differences in sites A and B support this idea. At site A where the habitat was more diverse, the number of empidid larvae found and the apparent impact of the predators on the midge population was significantly less than observed at site B. Perhaps the more diverse habitat at site A provided other prey and/or resting sites for the *Neoplasta* larvae but the more stark, rocky environment of site B provided little alternatives for the predators, and the impact on the midge population was greater.

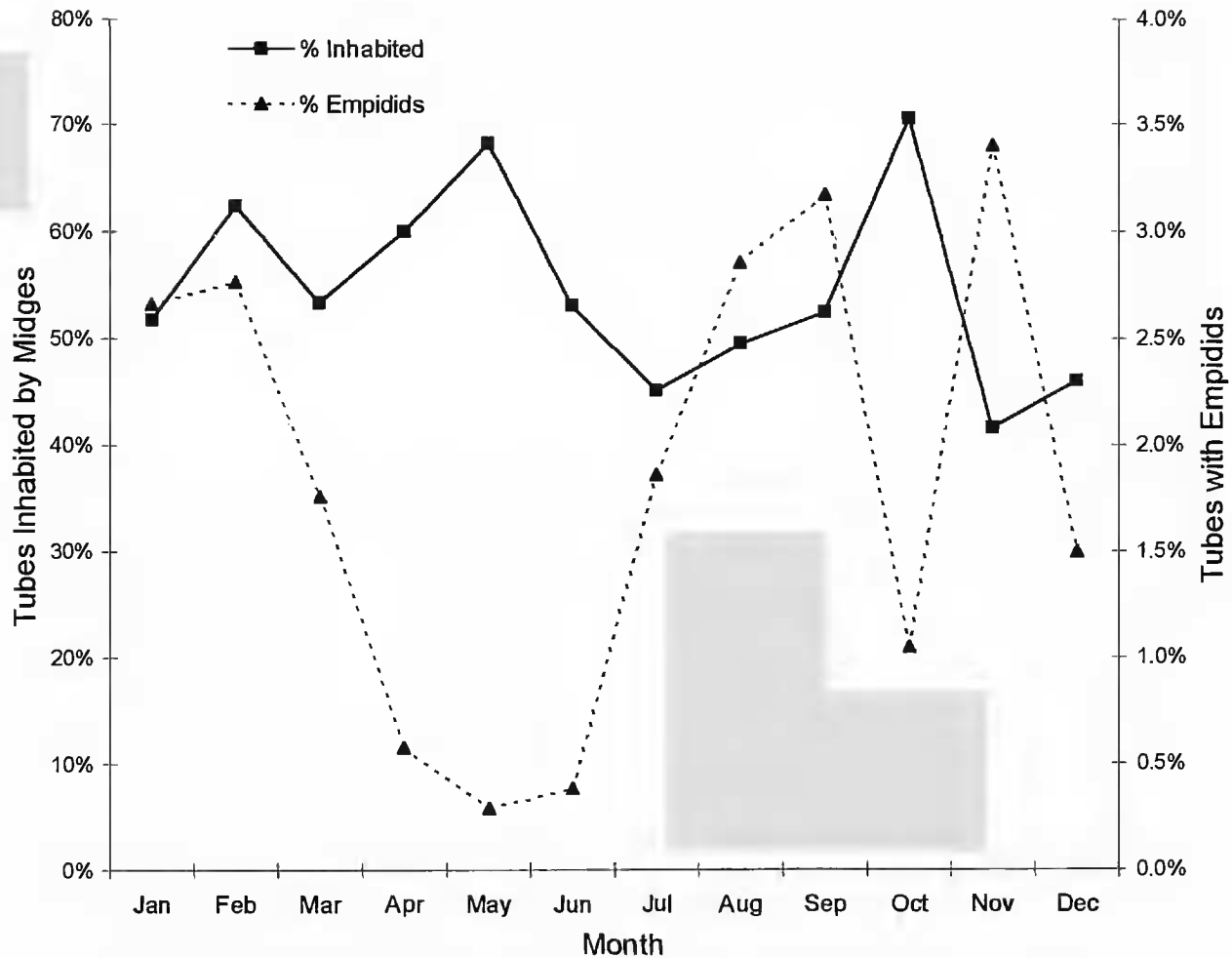The inverse relationship between the number of inhabited *Rheotanytarsus* tubes

Figure 4.   A summary of the percentage of *Rheotanytarsus* tubes inhabited by midges (either larvae or pupae) and the percentage of tubes containingempidid larvae collected (by month) at three Baldy Village collection site from October 1993, through August 1996.

and the number of empidids suggests a significant impact of the predators on the midge population (Fig. 4). It is not clear if this relationship is due solely to the mortality caused by predation or if abandonment of larval tubes may be a response of the midge larvae to *Neoplasta* larval activity. Other factors causing reduced habitation of the tubes, such as mermithid parasitism or pupation of the midge, are undoubtedly important, although little correlation existed between the number of pupae and percent inhabitants (Spearman cc = 0.192, P = 0.22). One might expect a negative correlation between the number of inhabited tubes and the number of pupae because a high level of pupation would suggest that tubes were being actively abandoned. Oddly, that was not the case. The midge tubes appeared to be short-lived. Once the inhabitants were no longer present to defend and maintain the tubes, the larval tubes quickly broke down. If this were not true, a population decline, such as seen in April 1994, would have been characterized by many of empty tubes rather than an absence of tubes. It was not.

Within the midge tube habitat, at least two species of *Rheotanytarsus* were present. Until the taxonomy of the host species is better defined, the population dynamics of these species and their relative importance remains an unknown.

The reduced host population from April through June (Figure 4) resulted in a scarcity of empidid larvae. Whether a decline in prey population resulted in a decline in predators, or the predators moved to a new prey population or the larval empidids began pupation and emergence in association with their prey remains unclear. Certainly, other habitats were important to empidid development. Although 4234 tubes were dissected and 74 tubes contained empidid larvae, not

a single empidid pupa was found in a midge tube. This strongly suggests that the empidids do not pupate in the midge tubes. Attempts to rear the empidids produced seven individuals that pupated in the rearing units. None pupated in midge tubes or in *Simulium* pupal cases that were made available; all pupated in the gravel. Knutson & Flint (1971) found *Neoplasta* pupae in Trichopteran cases of *Mortoniella apiculata* Flint in Chile. Similar trichopteran pupal cases were collected on several occasions at all three collection sites. Of a hundred or so specimens, a single larvae of *Neoplasta* was collected in association with a trichopteran pupal case, but no empidid pupae were found.

Figure 4 shows that larvae are most common from August through February but larvae are rare (less than 1% of tubes) from April through June. The period of scarcity coincides with the adult emergence (Harkrider 2000) which suggests that a single, annual generation occurs over a several months. This was similar to the long emergence period for *N. scapularis* described by Landry & Harper (1985).

There may be unique features to this particular habitat that support the *N. hebes* complex population. Surveys in other local habitats harboring *Rheotanytarsus* tubes produced larvae of *Roderoides wirthi* Chillcott, *Hemerodromia empiformis* Say, and *N. scapularis* in the tubes, but no *N. hebes* complex species. The small size, cryptic behavior, and relatively low density of these *Neoplasta* larvae make their study difficult. This study demonstrates that they play a role in the population dynamics of *Rheotanytarsus* species and possibly other species. On going study is needed to further elucidate the role of these empidids in the aquatic ecosystem.

ACKNOWLEDGMENT

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